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NITROGEN TRICHLORIDE AND OTHER GASES AS FUNGICIDES^{1, 2, 3}

L. J. KLOTZ⁴

INTRODUCTION

OBVIOUS ADVANTAGES are to be had in the use of a suitable gas for the control of fungi and insect pests on fruits in storage rooms and in cars during shipment. Application would be relatively simple and inexpensive; the material, because of its fluidity and diffusibility would, if aided by agitators, penetrate quickly to all exposed surfaces; and at the termination of the treatment, the gas could be readily eliminated by forced ventilation. Where tight refrigerator cars are used, it is possible that the fruit might remain in a low but effective concentration of the protective gas during shipment, assuring minimum losses from decay.

Nitrogen trichloride, the gas with which this report is chiefly concerned, is used extensively in the treatment of freshly milled flour in order to mature it quickly and induce desirable baking qualities. The suggestion that the gas might be used for the control of citrus pests was made several years ago when the Field Department Laboratory of the California Fruit Growers' Exchange and the Wallace and Tiernan Products Company began a series of coöperative experiments. At that time tests were made to determine whether or not the gas would decrease losses from the most serious organisms of decay in citrus fruits, namely, blue and green molds, or *Penicillium italicum* and *P. digitatum*. Such promis-

¹ Received for publication August 15, 1935.

² Paper No. 321, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

³ The results of this paper grew out of coöperative investigations with the staff of the Field Department Laboratory of the California Fruit Growers' Exchange and of the Wallace and Tiernan Products Company, Inc. To the staff of the former under the direction of A. E. Nelson, coöperating with J. C. Baker and T. E. Galvin of the latter, the author is indebted for facilities and data acknowledged later.

⁴ Associate Plant Pathologist in the Citrus Experiment Station.

ing experimental results with small lots of fruit were secured that trials with commercial carload lots and larger were made. The method has recently been adapted to the protection of fruit in storage rooms and refrigerator cars, and is now being used commercially by fifteen packing companies.

REVIEW OF PREVIOUS INVESTIGATIONS

A review of the literature reveals that several investigators have thought of the possibilities of gases as fungicides. Ludwig^(7, 8) grew a number of bacteria and fungi in several concentrations of illuminating gas, finding the organisms relatively insensitive to concentrations even as great as 25 per cent of gas in air. Studying the individual components of the illuminating gas, he found the toxicity of carbon monoxide about equal to, and that of methane and ethylene less than, that of the complete gas. Ethylene and methane showed scarcely any toxicity below a concentration of 40 per cent.

Oserkowsky⁽¹²⁾ found that at 30° C (86° F) the hyphae of *Sclerotium rolfsii* were killed by 2 to 4 days' exposure to air saturated with the vapors of any one of the following: trioxy-methylene, benzene, toluene, xylene, nitrobenzene, and the ortho, meta, and para forms of dichlorbenzene.

Neifert and Garrison⁽¹⁰⁾ showed that cyanogen chloride in concentrations of 3 per cent or greater for 2 hours was effective in killing species of *Fusarium*, *Ascochyta*, *Penicillium*, *Colletotrichum*, and *Sclerotium*.

Brooks *et al.*⁽⁹⁾ report that a concentration of 37 per cent carbon dioxide in air completely inhibited the growth of *Botrytis cinerea* and *Rhizopus nigricans* and decay of strawberries by these fungi. Thirty per cent or more of the gas prevented decay of prunes by *Sclerotinia fructicola* and of peaches by *B. cinerea*, and greatly inhibited decay of peaches due to *S. fructicola* and *R. nigricans*.

Brown⁽⁶⁾ showed carbon dioxide to be more inhibitive to spore germination than to hyphal growth, and to be more effective in retarding growth in water than in nutrient media, and at low rather than at high temperatures. He suggested a concentration of 10 to 20 per cent to reduce decay of apples in cold storage. Kidd, West, and Kidd⁽¹¹⁾ determined that an atmosphere containing 10 per cent carbon dioxide and 11 per cent oxygen was most suitable for apple storage.

Barker⁽²⁾ found that after 5 weeks at 1.1° or 4.9° C (34° or 41° F), 50 per cent carbon dioxide produced severe injury in African Navel orange, and that the injuries were aggravated by low oxygen content. On the other hand, where the oxygen content was sufficient for respiration

tion, high concentrations of carbon dioxide at 7.1° to 12.6° C (45° to 55° F) did not result in injury during the same period of storage. Thornton⁽¹⁹⁾ states, moreover, that the Foster Pink and Thompson varieties of grapefruit and Valencia oranges at a temperature range of 0° to 21° C (32° to 69.8° F) showed no injury after 7 days' exposure to 50 per cent carbon dioxide, but were injured by 65 per cent carbon dioxide; that the Walters grapefruit, King oranges, and tangerines withstood 25 per cent but were injured by 50 per cent of the gas.

Winkler and Jacob⁽²³⁾ and Jacob⁽⁵⁾ found that for grapes an absorption of 50 to 100 parts of sulfur dioxide per million parts of the fruit retarded the rate of deterioration about one-half. Pentzer, Asbury, and Hamner,^(13, 14) studying the rate of absorption of sulfur dioxide by several varieties of grapes, found that not more than 20 parts per million should be used for the varieties grown in the San Joaquin Valley. Pentzer and Asbury⁽¹⁵⁾ showed that for a period of 12 days, 5 parts per million in Sultanina grapes at 11.7° C (53° F) reduced decay from 20 per cent to less than 1 per cent; 46 parts per million gave complete protection but caused slight injury. Utilization of the sulfur dioxide slowly released by acid sodium sulfite in pads or sawdust packs showed promise.

Brief mention is here made of two papers on the use of formaldehyde vapor. To prevent potato seab, Morse⁽⁹⁾ treated seed potatoes with formaldehyde gas, using per 1,000 cubic feet of space (10 pounds of tubers per cubic foot) 3 pints of formalin with 23 ounces of potassium permanganate for vaporization. Thomas,⁽¹⁷⁾ in his work on the disinfection of seeds with formaldehyde vapor, was able to kill *Monilia fructigena*, *Ascochyta* sp., *Colletotrichum gloeosporioides*, and *Bacillus carotovorus* by steam vaporization of 20 ounces of formalin per 1,000 cubic feet for 2 hours. Four species of *Fusarium* survived the treatment.

Preliminary work by Trout and Tomkins⁽²¹⁾ indicates that the concentrations of acetaldehyde which will protect oranges from decay also cause injury to the rind of the fruit. With grapes, continuous storage in a concentration of 1/1,000 acetaldehyde markedly retarded the development of molds without apparent chemical injury. Short-period exposures (34 to 96 hours) to a 1/250 concentration at 25° C (77° F) were unsatisfactory, the shorter exposures not sufficiently checking mold growth and the longer exposures causing the development of an aldehyde taste in the fruit. At 1° C (33.8° F), however, grapes exposed 72 hours to a 1/250 concentration of the gaseous aldehyde were kept in good condition for 36 hours. The method also showed promise with cherries and prunes.

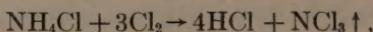
The same authors, Tomkins and Trout,⁽²⁰⁾ using ammonia solutions in

a system similar to that used for acetaldehyde, found that at 18° C (64.4° F) the introduction of volume concentrations of 5 to 10 parts per 10,000 lessened decay in wounded oranges inoculated with *Penicillium digitatum*. At lower temperatures, such as 10° C (50° F) and 3° C (37.4° F), volume concentrations as low as 1 part in 10,000 were effective. However, the lower temperatures also rendered the fruit more susceptible to ammonia injury. At 18° C (64.4° F) the rind would tolerate 10 parts per 10,000, but at 3° C (37.4° F) was injured at the wounds by a concentration of 3 parts per 10,000. Crystals of ammonium carbonate and ammonium bicarbonate were also found to supply a concentration of ammonia sufficient to lessen decay due to green mold, provided the conditions of storage were such that the crystals kept moist.

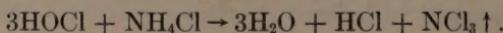
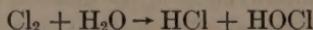
Tomkins⁽²⁰⁾ exposed agar cultures of *Trichoderma lignorum*, *Rhizopus nigricans*, *Thielaviopsis paradoxa*, *Gloeosporium musarum*, and *Botrytis cinerea* to several vapors and gases and compared the effects on germination and growth with the behavior of the fungi in air. The reactions of the several fungi were similar, and he confines the details of the report to *Trichoderma lignorum*. At 25° C (77° F) and in a closed container of 800-cc capacity, germination and growth of *T. lignorum* were greatly inhibited by quantities "greater than" 0.144 cc of hydrogen sulfide and 224 cc of hydrocyanic acid gas, and by the vapor from the following volumes of materials in respective 400 cc quantities of aqueous solutions: 0.2 cc chloroform, 2.4 cc acetal, 1.4 cc ethyl ether, 0.6 cc amyl formate, 12 cc ethyl alcohol, and 0.04 cc of concentrated ammonia solution. Germination was also inhibited by the vapor from 14 cc of acetone or 0.1 cc of acetaldehyde.

Wilcoxon and McCallan⁽²²⁾ found hydrogen sulfide very toxic to the conidia of *Sclerotinia americana*.

MANUFACTURE AND USE OF NITROGEN TRICHLORIDE
Chlorine and ammonium chloride are the materials employed in the generation of nitrogen trichloride. Empirically, the equation for the formation of the chemical may be written:



but according to J. C. Baker,⁵ who developed the method, the reaction probably proceeds in two steps, the hypochlorous acid formed by the reaction of chlorine and water reacting at once with the ammonium salt:



⁵ Personal interview, September 28, 1931.

Since gaseous NCl_3 is 4.18 times as heavy as air and readily collects in explosive droplets, it must be handled in dilution and cannot be accumulated in quantity and stored in metal cylinders for future use. To be handled safely it must be prepared with special apparatus which generates it as a gas highly diluted with air. The low concentrations effective

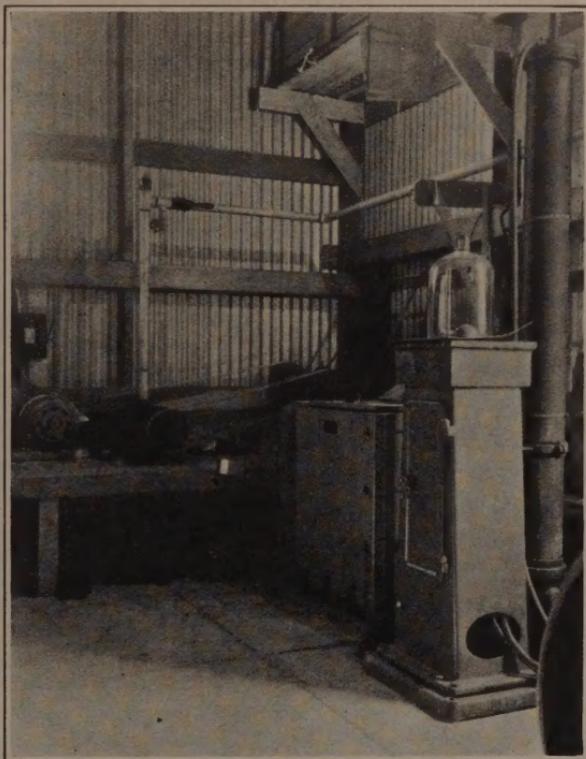


Fig. 1.—Single-stream NCl_3 generating unit used in experimental work.

for mold control are so irritating to the eyes and mucous membranes that the gas thus gives adequate warning before serious injury to humans can occur. With the equipment shown in figures 1 and 2, known quantities of the gas can be metered with known volumes of air; thus the concentration can be well controlled. However, for the safe and effective treatment of citrus fruits a trained and constantly attentive operator must be employed.

As applied to citrus fruits, the gas-air mixture is introduced into a refrigerator car or storage room and made to circulate rapidly by the use of electric fans. Determinations of the residual gas content of the

room are made from time to time by the use of a simple, rapid colorimetric method with orthotolidine as the indicator. By residual is here meant the concentration of the gas remaining in the air after the contents and walls of a room are saturated.

The procedure chosen in applying the gas treatment depends upon the manner of handling the fruit at the packing-house. There are three

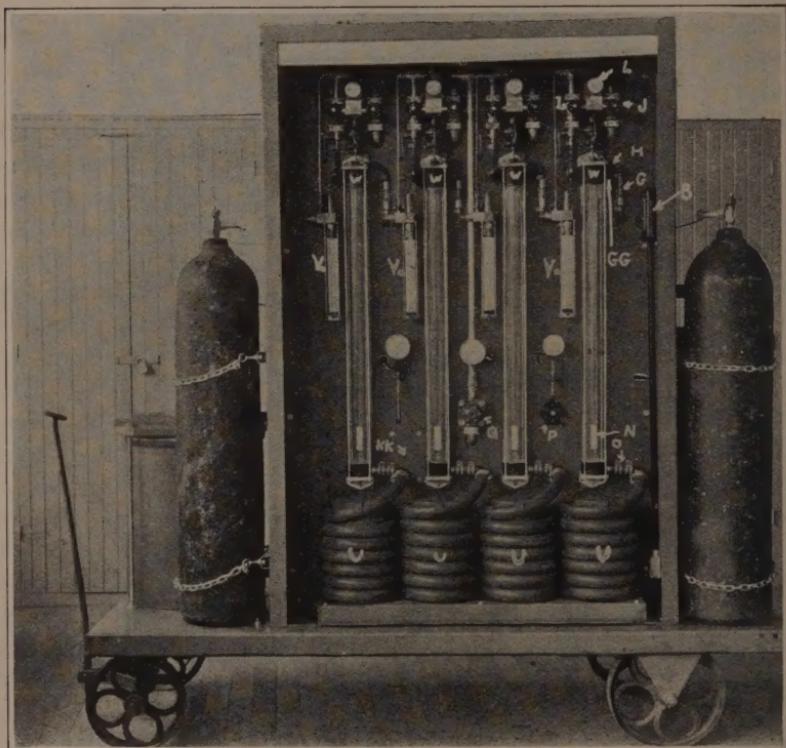


Fig. 2.—Four-stream NCl_3 generating unit used in citrus industry.

general procedures: namely, long storage, short storage, and no storage or the immediate packing-out of the fruit. In long storages a sterilizing treatment of from 1 to 3 applications of gas (8 mg NCl_3 per cubic foot) at intervals of 3 to 4 days is given when the fruit is first placed in the storage room. Additional gassings are made at weekly intervals to prevent reinfection. Just before removal of the fruit from storage another exposure is made to kill the fungus spores on any decay that may have developed, and to sterilize the field boxes in which the fruit was stored. This reduces the incidence of infection during the packing-house handling. Finally, the packed fruit is gassed in the refrigerator car to sterilize

ize any injuries that were made during the packing and loading operations and to protect the fruit in transit.

For fruit that is held in storage only a short time the same procedure is followed as for long-storage fruit. After the first sterilizing treatment, however, there would necessarily be fewer gassings during the shorter storage period.



Fig. 3.—Wounded Washington Navel oranges showing collapse and browning of wounds after three 6-hour experimental treatments with high concentration (40 mg per cubic foot) of NCl_3 . Such a high concentration is not used commercially.

Fruit that is not placed in the storage room, but is packed out and loaded at once, receives only the single gassing in the refrigerator car.

It should be noted here that the treatment with nitrogen trichloride may cause the rind tissues surrounding wounds to collapse and turn brown. The extent of such effect depends upon the concentration of and the duration of exposure to the gas. Figure 3 shows the result where an excessively high concentration of the gas was used. That such treated tissues offer unfavorable foci for decay was apparent early in the investigation.

EXPERIMENTS ON DECAY PREVENTION

Tests with Small Lots of Fruit.—Several experiments were conducted to determine the relative effectiveness of varying numbers of applications of gas in decreasing losses from decay due to *Penicillium italicum* and *P. digitatum*. Representative results are given in table 1. In the lots

called "injured and inoculated," Valencia oranges were wounded uniformly by a nail scratch on the shoulder before inoculation and treatment; in the lots marked "orchard run" the fruit received no wounds

TABLE 1

EFFECT OF NUMBER OF APPLICATIONS OF NITROGEN TRICHLORIDE IN CHECKING DECAY OF VALENCIA ORANGES DUE TO BLUE AND GREEN MOLDS, 1933
(226 fruits used in each lot)

Lot No.	Pretreatment	Gassing*		Percentage of decay after			
		Number of times	Intervals, days	2 weeks	4 weeks	6 weeks	10 weeks
First series; treatment begun September 22							
1	Injured and inoculated	0 (Soap check)	...	96.9	98.7	100.0	...
2		1	...	23.9	41.6	57.1	...
3		9	4	15.5	19.9	21.2	...
4	Orchard run, inoculated	0 (Soap check)	...	40.7	...	71.2	...
5		1	...	8.4	...	23.4	...
6		3	4	...	7.5	...	14.2
7†		3	4	...	4.9	...	14.6
8		16‡	4	...	0.0	...	21.2
Second series; treatment begun October 5							
9	Injured and inoculated	0 (Soap check)	...	86.7	...	99.5	...
10		0 (Soap check)	...	90.3	...	99.5	...
11		1	...	13.7	...	29.6	...
12		2	2	9.7	...	25.2	...
13		2	3	11.1	...	26.1	...
14		2	6	15.9	...	29.6	...
15		3	6	15.9	...	25.2	...
16		5	3	7.1	...	12.8	...
17		6	1	10.2	...	18.1	...
18		7	6	34.1	...
19		8	2	9.7	...	17.7	...
20		11§	1	18.6	...
21		13	3	16.8	...
22		16¶	2	14.2	...

* Concentration of gas: 27-50 mg NCl₃ per cubic foot. Exposure: 2-6 hours.

† Repetition of No. 6.

‡ Gassings on September 22, 26, 30, then at 4-day intervals from October 9.

§ Gassings at 1-day intervals October 5 to 10, then October 17, 25, 31, and November 6 and 14.

¶ Gassings at 2-day intervals October 5 to 21, then on October 25, 28, 31, and November 3, 6, 9, and 14.

prior to inoculation in addition to those already received in the orchard and in subsequent handling. Inoculation consisted of a single, quick "in and out" immersion of the fruit in a heavy spore suspension of the two molds.

Since the use of warm soap solutions to clean the fruits and lessen decay is a regular packing-house procedure, soap checks were used in

the experiments reported in table 1 for comparison with the gas treatments. "Soap check" means immersion for 1½ minutes in a ½ per cent soap solution at 46° C (115° F).

The results show that all the gas treatments of the oranges markedly lessened the decay. Lot 8, which had no visible decay at the end of 4 weeks, had received 7 gassings during that period, while lots 6 and 7, which had a decay of 7.5 per cent and 4.9 per cent respectively, had received but 3 gassings. At the end of 10 weeks, however, lot 8, which had then been given a total of 16 gassings, had 21.2 per cent decay, and lots 6 and 7, which had received no additional treatments, had only 14.2 per cent and 14.6 per cent respectively. This indicates that beyond a certain number of exposures fruit may not only receive no benefit from additional exposures, but may be actually adversely affected. Lot 16, which received but 5 gassings, developed less decay than lots that received more or fewer treatments. Early in the investigations, it was discovered that fruits which had received a large number of apparently noninjurious dosages of NCl_3 acquired an aged, shriveled appearance, indicating a loss of vitality and of resistance to organisms of decay.

Tests With Large Commercial Lots of Fruit.—To determine the protective effect of gas treatments on Washington Navel oranges under actual commercial storage conditions and in transit in refrigerator cars, coöperative experiments were conducted in Tulare County during the Washington Navel orange season of 1933–34. Since the results of these investigations have been reported by Nelson and Nedvidek,¹¹ only a brief reference is made to them here. The results showed the marked protective effect of the gas treatments. At the end of a month's storage the untreated fruit from an orchard that produced relatively weak, decay-susceptible oranges had 16.44 per cent decay, while the gassed fruit from the same orchard had 4.31 per cent decay. Three weeks after packing, the untreated lot of fruit had 28.2 per cent and the treated had 5.6 per cent additional fruits decayed. The average losses for the lots of fruit from 13 different orchards were 4.16 per cent for the untreated and 1.06 per cent for the treated fruit at the end of the storage period, and 16.3 per cent and 5.2 per cent respectively 3 weeks after packing.

On arrival in New York, 11 days after packing, 9 boxes of the gas-treated fruit and 9 of the check fruit were, through coöperative arrangement, examined by C. O. Bratley, of the United States Department of Agriculture. In addition, 2 boxes of freshly picked fruit (not stored) that had been included in the gassed car and 2 boxes of fresh unstored fruit included in the untreated car were also examined. Fruit from the

storage rooms showed 0.6 per cent decay in the check boxes and 0.3 per cent in the gassed boxes. After being held 10 days at 18.3° C (65° F) and 65 per cent relative humidity, the checks had 8.5 per cent decay and the gas-treated fruit 1.2 per cent decay. The check boxes of fresh fruit from the car that had received no gas showed 3.7 per cent decay on arrival and 30.3 per cent 10 days later, while similar fruit that had received the one gas treatment had 1.6 per cent on arrival and 10.9 per cent 10 days later.

The summarized conclusions of a large number of experiments on the NCl_3 treatment of oranges conducted as a co-operative project of the Field Department Laboratory of the California Fruit Growers' Exchange, the Citrus Experiment Station, and the Wallace and Tiernan Products Company, are given by Ramsey⁽¹⁰⁾ in a circular issued by the California Fruit Growers' Exchange. Since this circular is not available to the entire citrus industry, some of the conclusions are given here.

1. When blue contact mold is not as prevalent as common green mold, and the fruit is held in the packing-house for only 2 or 3 days before packing, treatment of the oranges with a solution of borax and boric acid under the approved commercial methods will generally retard the decay to as great an extent as the NCl_3 gas. If blue contact mold is prevalent, or the fruit is held for more than 3 days before packing and is gassed with the NCl_3 gas at the proper times, the NCl_3 gas treatment should show better results than a solution of borax and boric acid in reducing decay. The results secured with the gas have been obtained on experimental and semicommercial tests, and definite conclusions cannot be drawn until the process has been used for a year or two in some packing-house operating in the regular commercial manner.

2. Nitrogen trichloride gas in concentrations of 5 mg per cubic foot and upwards is lethal to the spores and mycelium of blue and green mold (*Penicillium italicum* and *P. digitatum*) and of various other fungi, such as *Alternaria citri*, *Colletotrichum gloeosporioides*, *Phytophthora citrophthora*, *Botryosphaeria ribis*, and *Oospora citri-aurantii*, the length of exposure to the gas being inversely related to the concentration of the gas.

3. The gas will penetrate the wrappers of a packed box of oranges loaded in a car and kill a large percentage of the mold spores on the fruit, provided there is sufficient air circulation. The use of two pre-cooling fans in a car will give sufficient air circulation.

4. With fruit shipped soon after picking, best results with the NCl_3 gas can be obtained by treating the oranges as soon as possible after they are picked, again the night before packing, and then after the packed

fruit is loaded in the car. The gas will kill the spores on the surface of an orange which has already decayed but cannot reach the mycelium within the rind. Gassing the fruit before decay starts is therefore essential.

5. When oranges have been held in coloring rooms or air-conditioned rooms before packing and decay has developed, a treatment with gas will kill most of the spores. Such a precautionary measure will reduce infection of sound fruit during washing and packing operations.

6. Because the NCl_3 gas is fatal to the spores whether they are on the fruit, on boxes, or in the air, continued use of the gas on all of the fruit in a packing-house should very materially reduce the spore load and thus reduce the chances of infecting sound fruit.

7. Too high a concentration of the gas or inadequate air circulation while it is being applied has resulted in a browning of injuries already on the fruit. This browning, which resembles pitting, can be readily avoided by adequate air circulation and an adequate control of the gas concentration by occasional tests and adjustments.

8. The NCl_3 gas has a tendency with most lots of fruit to retard color development, but this has generally been overcome by applying slightly higher doses of ethylene or by increasing the time of coloring 12 to 24 hours.

EFFECT OF NITROGEN TRICHLORIDE AND OTHER GASES ON CITRUS FUNGI

The following experiments accompanied those reported on decay prevention and were planned to determine the dosages of NCl_3 gas lethal to the several decay-producing organisms. Mention is made also of some experiments with chlorine, ozone, methylchloramines, and sulfur dioxide.

Comparison of Nitrogen Trichloride and Chlorine.—While more toxic to humans and consequently more dangerous to work in than NCl_3 gas, chlorine gas has the advantages of being nonexplosive and hence obtainable in metal cylinders which would permit easy application. Accordingly, it was advisable at the outset of the work to run some comparative tests of the two gases.

Figure 4 shows the results of 13 series of experiments⁶ in which four fungi were exposed for different periods of time to various concentrations of nitrogen trichloride and chlorine. The curves represent minimum lethal dosages, all time-concentrations on and above the curves being lethal and all below ineffective or only partially effective. It will be noted

⁶ The writer was assisted in this part of the work by E. C. Raby and subsequently by D. S. Giddings.

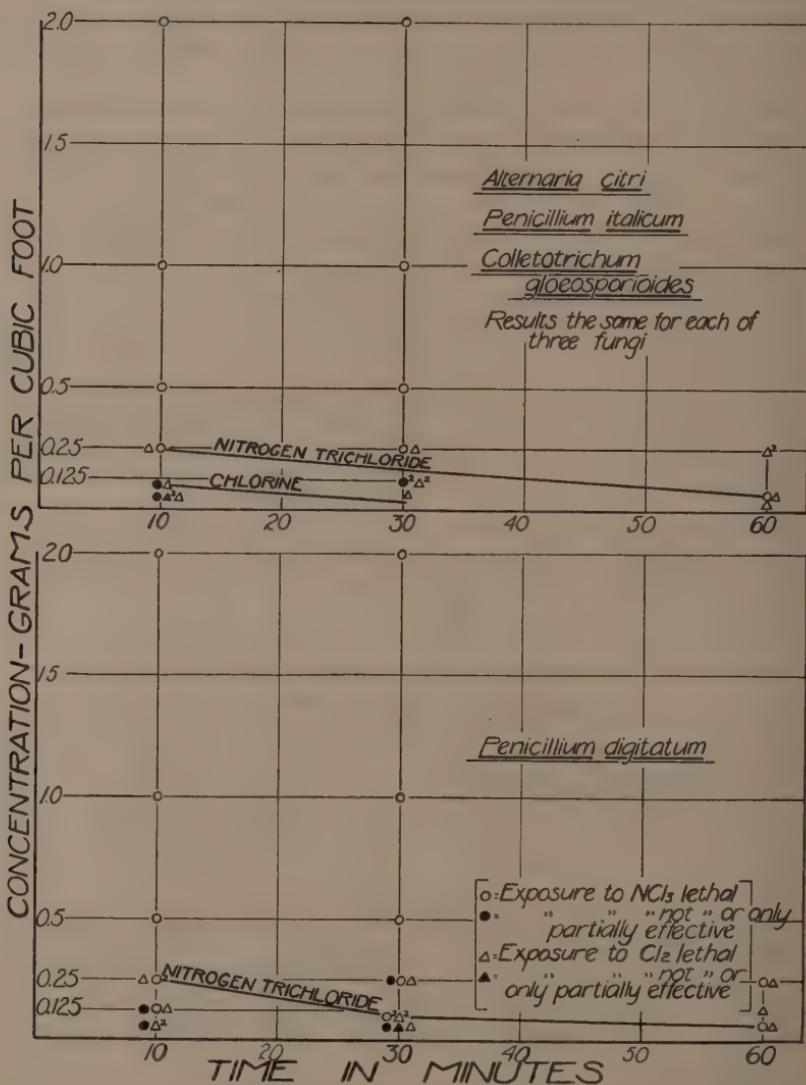


Fig. 4.—Lethal effect of several concentrations and exposures of nitrogen trichloride and chlorine upon four citrus fungi. The concentrations given here for the gases were those delivered to the treating chamber and do not represent the actual residual concentrations remaining in the air after the walls, shelves, etc., had been saturated with gas. Accurate means for the estimation of the concentration of the toxic gases in the air had not been perfected at this stage of the work.

that chlorine gas is more effective than nitrogen trichloride in killing pure cultures of the fungi.

However, trials on wounded and inoculated oranges, conducted thus far at the field laboratory of the California Fruit Growers' Exchange,

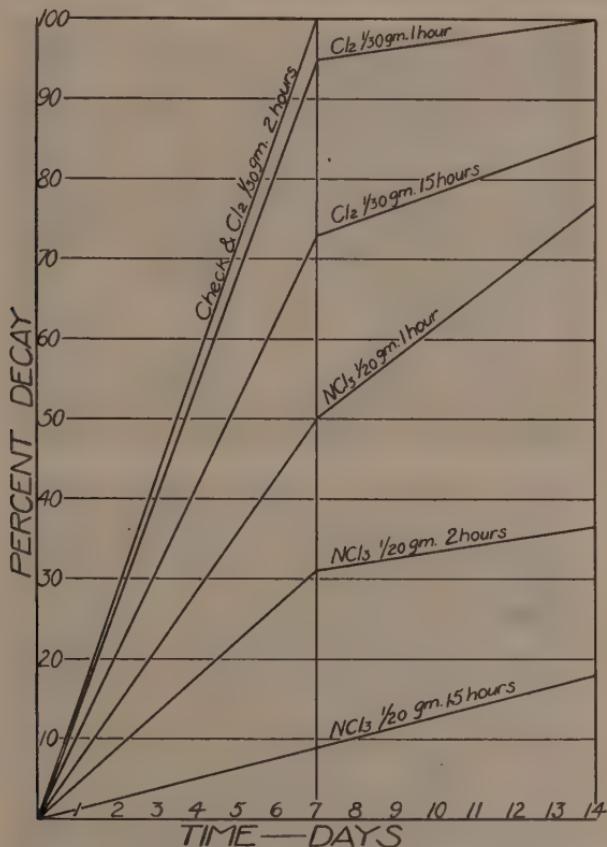


Fig. 5.—Comparison of nitrogen trichloride and chlorine in lessening decay in injured and inoculated Valencia oranges. The gases were delivered to the treating chamber in such concentrations that at the end of 15 minutes the concentrations shown were attained. The generator was then stopped and no additional gas introduced during the periods shown.

indicate that chlorine at the concentrations used was decidedly less effective than nitrogen trichloride in protecting the fruit from decay, the probable reason being that the chlorine injured the rind and made it more susceptible to the organisms of decay. This is shown in figure 5.

The concentration of $\frac{1}{30}$ gram of chlorine per cubic foot was used because it caused no rind injury apparent to the eye. The reactivity of gaseous chlorine is several times as rapid as that of nitrogen trichloride; that is, chlorine coming in contact with moist surfaces, such as those of

the protoplasm of citrus fruits and molds, would react with such violent rapidity that it would be much more apt to injure and kill the cells than would nitrogen trichloride, which releases its reactive chlorine less rapidly. Moreover, because of its great chemical reactivity, chlorine may



Fig. 6.—Above: Untreated plantings of *A*, *Colletotrichum gloeosporioides*; *B*, *Alternaria citri*; *C*, *Penicillium digitatum*; *D*, *Penicillium italicum*; *E*, *Phytophthora citrophthora*; *F*, *Botryosphaeria ribis*; after 4 days' incubation at 26° C. Below: Similar plantings treated exactly as those above but exposed to 30 mg per cubic foot of NCl_3 gas for 2 hours.

decrease in concentration below the point lethal to fungus spores before all the infected surfaces are contacted.

Nitrogen Trichloride.—The toxicity of NCl_3 gas to pure cultures of citrus fungi was apparent in the first series of experiments conducted. Figure 6 shows petri-dish cultures of *Colletotrichum gloeosporioides*, *Al-*

ternaria citri, *Penicillium digitatum*, *P. italicum*, *Phytophthora citrophthora*, and *Botryosphaeria ribis* that were left untreated as controls, and the corresponding six cultures that were killed by the gas.

Spore and mycelial suspensions of the organisms were made by pouring sterile water on agar slant cultures and loosening the growth with a



Fig. 7.—Effect of NCl_3 on conidia (c) and germ tubes (gt) of several citrus fungi; A, control conidium and germ tube of *Alternaria citri*; B and C, NCl_3 -gas-treated conidia and germ tubes of *Alternaria citri* showing shrunken protoplasts (A, B, C $\times 600$); D, untreated germinating conidia of *Penicillium italicum*; E, F, and G, gas-treated *Penicillium italicum* with shrunken protoplasts; H and I, untreated conidia, germ tubes, and appressoria of *Colletotrichum gloeosporioides*; J, gassed, ungerminated conidia of *Colletotrichum gloeosporioides* (D-J, $\times 260$).

platinum needle. Three drops of the suspension were placed aseptically on a sterile filter paper in a petri dish. After the closed dishes had been exposed to the gas for 5 minutes, the lids were set so as to cover a half or less of the bottom parts of the dishes, a rapid diffusion of the gas to the fungi thus being permitted. After treatment the cultures were returned to the laboratory and tested for viability by pouring on them melted glucose-potato agar at 45°C (113°F). The amount of, or the absence of, subsequent growth was the criterion of the effectiveness of treatment.

TABLE 2
LETHAL EFFECT* OF THREE CONCENTRATIONS OF NCl_3 ON CONDIA AND MYCELIUM OF CITRUS FUNGI†

Concentration of NCl_3 , mg per cu. ft.	Exposure, hours	<i>Phytophthora</i> <i>citrophthora</i>				<i>Colletotrichum</i> <i>glycosporioides</i>				<i>Alternaria</i> <i>citri</i>				<i>Penicillium</i> <i>digitatum</i>				<i>Penicillium</i> <i>italicum</i>				
		Dry		Wet		Dry		Wet		Dry		Wet		Dry		Wet		Dry		Wet		
		—	—	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0 (checks)
6 25	...	3	—	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6 25	...	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
31 00	...	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
31 00	...	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
105 00	...	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
105 00	...	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

* Explanation of symbols: + = growth; \pm = growth, but much less than checks; — = no growth. Number of signs = number of petri-dish cultures tested.

† Results 48 hours after agar poured on treated fungi.

Microscopically the protoplasts of the spores, germ tubes, and hyphae treated with nitrogen trichloride were seen to be shrunken and pulled away from the cell walls; in some cases marked granular and coagulation effects were evident. These effects are shown in figure 7.

To determine the effect of three relatively low concentrations of NCl_3 gas on five fungi that commonly attack citrus fruit, conidia and hyphae were placed on filter papers in petri dishes and exposed for 3 and 6-hour periods as shown in table 2. "Dry" means that the fungus was transferred directly without wetting from the test-tube cultures to the filter papers; "wet" means that a suspension of conidia and hyphae in water was the inoculum used.

Since the gas is only very slightly soluble in water, it was thought that possibly the water of the suspensions might protect the fungi. The results, however, show no such protection. In fact, the only evidences of growth were in 3 dry cultures. As a possible explanation of this incomplete killing, may be considered the fact that when the fungus is transferred dry, it is impossible to avoid hyphal and conidial clumps. These prevent an even distribution of the fungus and protect some cells from exposure to the gas. In the wet cultures the fungus is more easily broken up and distributed throughout the suspension. When the suspension is transferred, the water is quickly taken up by the filter paper, leaving the fungus unprotected and readily contacted by the gas. All the conidia planted on agar slants except those in the condensation water at the bottom of the slant are readily killed by the gas. Those protected by the water germinate and quickly cover the surface of the slant. It should be pointed out here that the surfaces of fruits when treated should be free from liquid water. The condensation water that forms on the rind of oranges coming from the precooler lessens the effectiveness of the gas treatment by preventing contact with some fungus spores.

Table 2 indicates that for a 3-hour period the minimum lethal dosage is less than 6.25 mg of the gas per cubic foot.

To determine the shortest exposure to a low concentration of NCl_3 gas that will kill *Penicillium digitatum* and *P. italicum*, plantings of water suspensions of these fungi on filter paper were exposed to a concentration of 4.75 mg of NCl_3 per cubic foot for the periods shown in table 3.

The data in table 3 indicate that an exposure of 20 or more minutes to the low concentration of 4.75 mg per cubic foot of NCl_3 gas was lethal to the hyphae and conidia of *Penicillium italicum*. Exposures of 60 to 90 minutes were required to kill all the viable cells of *P. digitatum*, although some plates showed no growth of *P. digitatum* after a 20-minute exposure. The conidia of *P. digitatum* are less easily loosened from the

conidiophores than are those of *P. italicum*, which necessitates a more vigorous manipulation with the platinum needles and loosens more clumps of mycelium. Cells in the interior of these clumps are less apt to be contacted and killed by the gas.

Work similar to that discussed under table 3 was carried on with *Colletotrichum gloeosporioides* and *Alternaria citri* and was repeated with the two *Penicillium* species.

TABLE 3

EFFECT ON GROWTH* OF *PENICILLIUM* spp. OF VARIOUS EXPOSURES TO 4.75 MG PER CUBIC FOOT OF NCl_3 GAS

Duration of exposure, minutes February 5, 1934	<i>Penicillium digitatum</i>		<i>Penicillium italicum</i>	
	February 6, 1934	February 8, 1934	February 6, 1934	February 8, 1934
10.....	± 3 col.	± 3 col.	± 1 col.	± 16 col.
20.....	—	± 1 col.	—	—
30.....	—, —	± 1 col., —	—, —	—, —
40.....	± 3 col.	± 3 col.	—	—, —
50.....	± 1 col.	± 1 col.	—	—
60.....	± 1 col.	± 1 col.	—	—
80.....	—	—	—	—
120.....	—	—	—	—
150.....	—	—	—	—
180.....	—	—	—	—
Checks†.....	+, +, +, +, +	+, +, +, +, +	+, +, +, +, +, +	+, +, +, +, +, +, +

* Explanation of symbols: + = growth; ± = growth, but much less than checks; — = no growth. Number of signs = number of petri-dish cultures tested.

† Checks had 50 or more colonies each.

The data of table 4 show that the *Penicillium* species, blue and green molds, are more easily killed by gassing than are *Alternaria citri* and *Colletotrichum gloeosporioides*. In making a spore suspension of the latter two fungi, it is very difficult or impossible not to include large clumps of mycelium, cells in the interior of which would be protected from the gas. With the *Penicillium* species, spore suspensions free from such clumps are more easily prepared. The data under "Conidial germination" in table 4 indicate that the spores of *Alternaria citri* and *Colletotrichum gloeosporioides* are likewise easily killed, provided the gas can contact them.

The success of the gassing method on Washington Navel and Valencia oranges led to attempts to adapt the procedure for the protection of lemons and grapefruit in storage and in transit. The latter two fruits are much more subject to injury by the gas than are oranges, which necessitates the use of lower concentrations. This injury is generally in the form of very minute pits which are rather noticeable on the light-colored rind of lemons and grapefruit. While the same type of injury

TABLE 4
EFFECT ON GROWTH* AND GERMINATION OF CITRUS FUNGI OF VARIOUS EXPOSURES TO 7.7 MG PER CUBIC FOOT OF NCl_3 GAS

Duration of exposure, minutes	Culture No.	<i>Colletotrichum gloeosporioides</i>		<i>Alternaria citri</i>				<i>Penicillium digitatum</i>				<i>Penicillium italicum</i>			
		2 days	4 days	Conidial germination after 4 days	Mycelial growth after 2 days	Mycelial growth after 4 days	Conidial germination after 4 days	Mycelial growth after 2 days	Mycelial growth after 4 days	Conidial germination after 4 days	Mycelial growth after 2 days	Mycelial growth after 4 days	Conidial germination after 4 days		
10	{ 1	—	—	—	—	—	—	—	—	—	—	—	—	—	
	{ 2	—	—	+ 3 col.	—	+ 1 col. + 2 col.	—	—	—	—	—	—	—	—	
20	{ 1	—	—	+ 1 col.	—	—	—	—	—	—	—	—	—	—	
	{ 2	—	—	+ 1 col.	—	—	—	+ 1 col.	—	—	—	—	—	—	
30	{ 1	—	—	—	—	—	+ 1 col.	—	—	—	—	—	—	—	
	{ 2	—	—	—	—	—	+ 1 col.	—	—	—	—	—	—	—	
40	{ 1	—	—	—	—	—	+ 1 col.	—	—	—	—	—	—	—	
	{ 2	—	—	—	—	—	+ 1 col.	—	—	—	—	—	—	—	
50	{ 1	—	—	+ 1 col.	—	—	—	—	—	—	—	—	—	—	
	{ 2	—	—	+ 1 col.	—	—	—	—	—	—	—	—	—	—	
60	{ 1	—	—	+ 1 col.	—	—	—	—	—	—	—	—	—	—	
	{ 2	—	+ 1 col.	—	—	—	+ 1 col.	—	—	—	—	—	—	—	
90	{ 1	—	—	+ 1 col.	—	—	+ 1 col.	—	—	—	—	—	—	—	
	{ 2	—	—	+ 1 col.	—	—	+ 2 col.	—	—	—	—	—	—	—	
120	{ 1	—	—	—	—	—	+ 2 col.	—	—	—	—	—	—	—	
	{ 2	—	—	—	—	—	+ 3 col.	—	—	—	—	—	—	—	
150	{ 1	—	—	+ 1 col.	—	—	—	—	—	—	—	—	—	—	
	{ 2	—	—	+ 1 col.	—	—	+ 1 col.	—	—	—	—	—	—	—	
180	{ 1	—	—	+ 1 col.	—	—	+ 1 col.	—	—	—	—	—	—	—	
	{ 2	—	—	+ 2 col.	—	—	+ 3 col.	—	—	+ 30 col.	—	—	—	—	
Checks	{ 1	+	—	+ 3 col.	+	—	+ 3 col.	+	+	+ 30 col.	+	+	+	+	
	{ 2	+	—	+ 3 col.	—	—	+ 3 col.	+	+	+ 30 col.	—	+	+	+	
	{ 3	+	—	+ 3 col.	—	—	+ 3 col.	+	+	+ 30 col.	—	+	+	+	
	{ 4	+	—	+ 3 col.	—	—	+ 3 col.	+	+	+ 30 col.	—	+	+	+	

* Explanation of symbols: — = no growth or germination; + = growth or germination; col. = colony; ± = slight growth.

TABLE 5
EFFECT* OF A VERY LOW CONCENTRATION (1.25 MG PER CUBIC FOOT) OF NCl_3 ON GERMINATION AND GROWTH OF CITRUS FUNGI, AUGUST 19, 1934†

Duration of exposure, hours	<i>Colletotrichum gloeosporioides</i>		<i>Alternaria citri</i>		<i>Oospora citri-aureantii</i>		<i>Penicillium digitatum</i>		<i>Penicillium italicum</i>	
	Germination	Growth on agar	Germination	Growth on agar	Germination	Growth on agar	Germination	Growth on agar	Germination	Growth on agar
3.....	—	—	+	+	—	—	—	—	—	—
6.....	—	—	±	+	—	—	—	—	—	—
12.....	—	—	—	+	—	—	—	—	—	—
24.....	—	—	—	—	—	—	—	—	—	—
0 (check).....	+	+ 3 col. or more	+ 3 col. or more	+ 3 col. or more	+ 50 col. or more	+ 50 col. or more	+ 50 col. or more	+	+ 50 col. or more	+ 50 col. or more

* Explanation of symbols: + (with mycelia) = growth, + (with spores) = germination more than 50 per cent; ± = germination 1 to 10 per cent; — = germination less than 1 per cent.

† Results were recorded 48 hours after treatment.

occurs to some extent on oranges, it is scarcely discernible because of the darker color of the rind of that fruit. In addition to their tendency to pitting, lemons and grapefruit are more subject than oranges to a staining and brown discoloration due to overdoses of the gas. The work with lemons and grapefruit is still in the experimental stages. Table 5 indicates the effectiveness of a very low concentration of nitrogen trichloride (1.25 mg NCl₃ per cubic foot) on several citrus fungi. If it is found that lemons and grapefruit will tolerate such low concentrations of the gas for long periods, gas treatments should prove of great benefit in decreasing losses from decay of these fruits in storage.

Ozone.—The possibility of utilizing ozone as a sterilizer and protector of citrus fruits was also considered. This, however, was unsatisfactory because it was found that the gas at the concentrations⁷ attainable with a small generator in a small chamber (3.646 cubic feet) at best only partially inhibited the germination and growth of the fungi on agar. When the cultures were removed from the gas chamber, even after 3 weeks' exposure, they resumed their usual rapid rate of growth. While in the presence of the gas, the fungi grew slowly within the medium and closely appressed to the surface, generating an alcoholic or ester-like odor and suggesting anaerobiosis. The cultures gave the iodoform reaction.⁸

Washington Navel oranges injured and then inoculated by immersion in a suspension of spores of *Penicillium digitatum* and *P. italicum* were not protected from decay when placed in ozone. The fungi failed to produce the characteristically colored fructifications while in the gas, except where the decayed rind was closely appressed to the floor of the gas chamber. However, the short, white, aerial hyphae destined to be conidiophores were in evidence on the surface of the fruits while in the presence of ozone, and within 16 hours after the stopping of the generator and the aeration of the chamber, the colored spores were in evidence. After 4 days at 25° C (77° F) the average diameter of the decayed area on the surface of fruits exposed to ozone was 103 mm, while the area on fruits allowed to decay in air was 114 mm, indicating but slight inhibition of the decay by the gas. Accordingly it was concluded that ozone in concentrations decidedly unpleasant and probably toxic to humans does not protect citrus from decay by the common blue and green molds.

Baker¹⁰ obtained similar results with apples. His work indicated that

⁷ The actual concentration of O₃ was not determined. It was sufficiently high, however, to give a strong, unpleasant odor and to cause headache, indicating toxicity to humans.

⁸ Indicates presence of such substances as alcohols, acetone, or aldehydes.

TABLE 6
EFFECT* OF OZONE AND NITROGEN TRICHLORIDE ON CITRUS FUNGI (GERMINATION)†

Gas	Concentration, mg per cu. ft.	Hours exposed	<i>Colletotrichum gloeosporioides</i>		<i>Alternaria citri</i>		<i>Penicillium digitatum</i>		<i>Penicillium italicum</i>	
			Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Checks.....	+, +	+, +	+, +	+, +	+, +	+, +	+, +	+, +
Ozone.....†	{ 3 6 9	+	+	+	+	±	+	±	+
N ₂ Cl ₃	6.25 6.25 15.00	3 6 2	-	-	-	-	-	-	-	-

* Explanation of symbols: + = more than 50 per cent germination; ± = less than 5 per cent germination; - = no germination.

† Concentration unknown; small generator run continuously in chamber of 12-cubic-foot capacity.

‡ Results 48 hours after treatment.

ozone has no effect on the appearance, general condition, flavor, and keeping quality of apples in storage.

Comparison of Ozone and Nitrogen Trichloride.—The results of an experiment designed to compare the relative effectiveness of ozone and nitrogen trichloride are shown in table 6. Both dry spores from an agar slant culture and the wet spores of a water suspension were exposed and the effect on germination noted.

The concentration of the ozone was unknown, there being no dependable quantitative method of estimation available at the time. However, a comparison of ozone with the relatively low concentrations of nitrogen trichloride given in tables 2, 3, 4, 5, and 6 shows the relative ineffectiveness of the former gas. Only on the dry spores of the *Penicillium* species did the ozone give any evidence of inhibition of germination.

Methylchloramines.—Experiments with two other gases, monomethylchloramine (CH_3NCl_2) and dimethylchloramine ($[\text{CH}_3]_2\text{NCl}$), which presumably are less corrosive than NCl_3 and therefore more easily adaptable to the treatment of lemons and grapefruit, are being initiated. Preliminary tests with CH_3NCl_2 indicate that it is at least as effective as and possibly superior to NCl_3 in sterilizing the surface of fruit. The cost of preparing CH_3NCl_2 is more than that of NCl_3 , however. A concentration of 20 mg of CH_3NCl_2 per cubic foot for periods of 1 and 2 hours was lethal to *Penicillium italicum* and *Alternaria citri*.

Sulfur Dioxide.—Several packing-houses are using sulfur dioxide gas for sterilization of used boxes and for general disinfection purposes to lessen the hazards of reinfection, principally by brown rot and blue and green molds. Experiments are now in progress to determine the minimum concentrations of the chemical lethal to several citrus fungi. Exposure to a volume concentration of 1 per cent SO_2 in air for 10 minutes was lethal to the brown rot fungus, *Phytophthora citrophthora*, but not to the blue and green molds, *Penicillium italicum* and *P. digitatum*. However, volume concentrations of 2 per cent or greater for 10 minutes were lethal to the two *Penicillium* species. Thus far no combinations of concentration of SO_2 and period of exposure have been found which, without injuring the rind, will protect oranges from decay.

SUMMARY

1. A gaseous mixture of low concentrations of nitrogen trichloride with air has been used successfully in controlling decay of Washington Navel and Valencia oranges due to *Penicillium italicum* (blue mold) and *P. digitatum* (green mold).
2. Procedures for the safe and effective use of the gas treatment in

storage rooms and refrigerator cars have been developed and commercial installations of apparatus have been made in the plants of fifteen packing companies.

3. After 3 to 4 weeks' storage, losses in oranges due to decay, as shown by numerous tests, may be reduced 50 to 75 per cent or more by giving the fruit three to five 3-hour treatments with 5 to 15 mg of NCl_3 per cubic foot of air, at 3 or 4-day intervals, beginning the first day of storage.

4. Concentrations as low as 4 to 6 mg NCl_3 per cubic foot for a period of 30 minutes were lethal to conidia of *Penicillium italicum*, *P. digitatum*, *Oospora citri-aurantii*, *Colletotrichum gloeosporioides*, *Alternaria citri*, and *Botryosphaeria ribis*, and to the mycelium of *Phytophthora citrophthora*.

5. Microscopically, the protoplasts of the gas-treated spores, germ tubes, and hyphae were seen to be shrunken away from the cell walls, in some cases with marked granular and coagulation effects.

6. Preliminary trials indicate that the method may be adapted to the treatment of grapefruit and lemons in storage by the use of lower concentrations of the gas for long periods.

7. Chlorine used in the same concentrations as nitrogen trichloride was injurious to the fruit and much less effective than the latter in decreasing decay. It was, however, more toxic to the fungi in cultures, and may be used for general disinfection of packing-houses and equipment.

8. Ozone showed only very slight or no toxicity to the organisms of decay and no protection of the fruit.

9. Preliminary tests indicate that monomethylchloramine may be substituted for nitrogen trichloride, but at greater cost.

10. Sulfur dioxide may be used effectively in the sterilization of boxes and for general disinfection purposes in the packing-house.

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